Diversity of Epidemic Populations of *Gibberella zeae* from Small Quadrats in Kansas and North Dakota

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Accepted for publication 1 March 2003.

ABSTRACT

Zeller, K. A., Bowden, R. L., and Leslie, J. F. 2003. Diversity of epidemic populations of *Gibberella zeae* from small quadrats in Kansas and North Dakota. Phytopathology 93:874-880.

Gibberella zeae (anamorph *Fusarium graminearum*) causes Fusarium head blight (FHB) of wheat and barley and has been responsible for several billion dollars of losses in the United States since the early 1990s. We isolated *G. zeae* from the top, middle, and bottom positions of wheat spikes collected from 0.25-m^2 quadrats during severe FHB epidemics in a single Kansas (KS) field (1993) and in a single North Dakota (ND) field (1994). Three amplified fragment length polymorphism (AFLP) primer pairs were used to resolve 94 polymorphic loci from 253 isolates. Members of a subset of 26 isolates also were tested for vegetative compatibility groups (VCGs). Both methods indicated high levels of genotypic variability and identified the same sets of isolates as probable clones. The mean number of AFLP multilocus haplotypes per head was approximately 1.8 in each population, but this value probably underestimates the true mean due to the small number of samples taken from each head. Isolates with the same AFLP haplotype often were recovered from different positions in a single head, but only rarely were such apparently clonal isolates recovered from more than one head within a quadrat, a pattern that is consistent with a genetically diverse initial inoculum and limited secondary spread. The KS and ND samples had no common AFLP haplotypes. All *G. zeae* isolates had high AFLP fingerprint similarity (>70%, unweighted pair group method with arithmetic means similarity) to reference isolates of *G. zeae* lineage 7. The genetic identity between the KS and ND populations was >99% and the estimated effective migration rate was high ($Nm \approx 70$). Tests for linkage disequilibrium provide little evidence for nonrandom associations between loci. Our results suggest that these populations are parts of a single, panmictic population that experiences frequent recombination. Our results also suggest that a variety of population sampling designs may be satisfactory for assessing diversity in this fungus.

Additional keywords: scab, *Triticum aestivum*.

Gibberella zeae (Schwein.) Petch (anamorph *Fusarium graminearum* Schwabe) causes Fusarium head blight of wheat and barley (30), stalk rot and ear rot of maize (57), seedling blight and stalk rot of sorghum (18), and stalk and ear rot of rice (42). *G. zeae* and *G. coronicola*, which causes dryland foot rot or crown rot (16), recently were separated taxonomically (1,2). In the last decade, *G. zeae* has caused destructive epidemics on wheat and barley in the United States (30) and Canada (20), with cumulative losses estimated at greater than $3 billion (58). Severe Fusarium head blight epidemics also have been reported in East Asia (10,35), South America (45), and Europe (4).

In addition to direct yield losses, Fusarium head blight causes discolored and shrunken grain (scab), and reduces wheat end-use quality (22,40). The harvested grain may be contaminated with mycotoxins such as deoxynivalenol, nivalenol, or zearalenone (4,22,35) that pose health risks to both humans and domesticated animals (27,46) and make it difficult to market scabby wheat. Although progress is being made in disease reduction through chemical control, cultural control, and the development of resistant host cultivars, satisfactory levels of control have yet to be attained (20,30,58).

An important component of the effort to manage this pathogen is knowledge of its population genetic structure. Understanding the genetic structure of pathogen populations may provide insights into the epidemiology and evolutionary potential of *G. zeae*, and could lead to improved strategies for controlling this fungal pathogen. There is growing evidence for genetic differentiation in *G. zeae* based on the toxins produced (e.g., nivalenol and deoxynivalenol) (21), and on DNA sequences (41). Of the eight lineages identified following DNA sequencing (55), all can cause Fusarium head blight, and at least some of these phylogenetic lineages are sexually cross-fertile under laboratory conditions (7,23). Within North American field collections, only lineage 7 has been detected (41).

G. zeae is homothallic and produces perithecia under both laboratory (7,39) and field (3,17) conditions. An outcross between strains of *G. zeae* from different lineages was used to produce progeny from which a genetic linkage map of *G. zeae* was constructed (23). Outcrossing has not been demonstrated in field populations, but has been inferred from studies using vegetative compatibility groups (VCGs) (5,35) and molecular markers (14, 34,50). Even relatively low rates of outcrossing can have a significant impact on population structure (26,52), and on gene flow among populations, because ascospores are thought to be an important means of natural dispersal for *G. zeae* (3,17,51).

Our objective in this study was to assess genetic diversity within very small (0.25-m^2) quadrats from single, widely separated sites in Kansas (KS) and North Dakota (ND), and to determine whether there was significant genetic differentiation due to the geographic origin of the strains. From these data, we could determine if the known high levels of regional genetic diversity reported previously (5,14,35,43,50,54) were observed at a very small scale within a field, and whether single wheat heads were infected by multiple strains of *G. zeae*. We also used data from a

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Publication no. P-2003-0508-03R

set of genetically mapped amplified fragment length polymorphism (AFLP) markers (23) to determine if there was evidence for genetic disequilibrium in either of the populations. Preliminary reports of some of these results have been published (6,59).

MATERIALS AND METHODS

Sample collection. The first quadrat sample was collected from a mature commercial wheat field during a naturally occurring scab epidemic on 15 July 1993 near Holton, KS. The wheat cultivar in the field was unknown. Harvest in the area had been delayed by excess rain, which allowed development of *G. zeae* perithecia on the wheat heads. Approximately 90% of the heads had symptoms of Fusarium head blight. The field had previously (1992) been planted in sorghum, but there was very little sorghum residue on the soil surface at the time of sampling. The second quadrat sample was collected by M. P. McMullen from a mature commercial wheat field during a naturally occurring scab epidemic on 20 August 1994 near Fargo, ND. The wheat cultivar in the field was Grandin, and disease incidence was estimated as >70%. In both fields, a 0.5-by-0.5-m quadrat was established ≈25 m from one edge of the field. In all, 50 to 54 wheat heads were selected arbitrarily from within the quadrat, placed individually in loosely closed plastic bags to prevent cross contamination, and dried at room temperature.

We sampled individual spikelets from a top, middle, and bottom position of each head. Two seed were removed from spikelets at each of these positions and surface disinfested in 0.5% sodium hypochlorite for 20 to 30 s, rinsed in 70% ethanol, and then blotted dry on clean paper towels. Seed were plated individually onto a semiselective peptone-pentachloronitrobenzene medium (36). As *Fusarium* colonies grew out of the seed, one randomly chosen colony was subcultured per spikelet onto complete medium (13). All isolates were purified by separating macroconidia with a micromanipulator, and cultures derived from individual macroconidia were used for further analyses. The resulting strains were preserved as spore suspensions in 15% glycerol and frozen at –70°C.

VCGs. Nitrate nonutilizing (*nit*) mutants of isolates from 10 wheat heads from the Kansas quadrat were obtained and tested for vegetative compatibility as described previously (5).

DNA manipulation and AFLP methodology. Mycelia were cultured and harvested and DNA extracted as described in Kerényi et al. (24). Extracted DNA was resuspended in 50 to 100 µl of 1× Tris-EDTA buffer and stored at –20°C until used. DNA concentrations were estimated densiometrically against a known concentration of *Hin*dIII-digested bacteriophage λ DNA (Promega Corp., Madison, WI) with an IS-1000 digital imaging system (Alpha Innotech Corp., San Leandro, CA).

AFLPs (53) were generated as modified by Zeller et al. (60). We used all buffers and DNA modifying enzymes following manufacturer's instructions or standard protocols (49). AFLPs were generated for all isolates with the specific primer pair combinations *Eco*+AA/*Mse*+AT, *Eco*+CC/*Mse*+CG, and *Eco*+TG/*Mse*+TT. These primer pairs were among those used by Jurgenson et al. (23) to generate a genetic linkage map for *G. zeae*. The *Eco*RI primers used in final specific polymerase chain reaction amplifications were end-labeled with γ^{33}P-ATP, and the resulting DNA fragments separated in 6% denaturing polyacrylamide (Long Ranger, FMC Scientific, Rockland, ME) gels in 1× Tris-borate EDTA buffer (pH 8.3). We exposed dried gels to autoradiography film (Classic Blue Sensitive, Molecular Technologies, St. Louis) for 2 to 7 days at room temperature to expose AFLP banding patterns. Band sizes were estimated on polyacrylamide gels against γ^{33}P-labeled BRL low-mass ladder (Life Technologies, Rockville, MD).

We manually scored the presence or absence of polymorphic AFLP bands ranging from 100 to 800 bp in length, and recorded the data in a binary format. All polymorphic bands in this size range were scored, including those found in only a single isolate. Bands with the same mobility from different individuals were assumed to be homologous and to represent the same allele. Each scored band of differing mobility was treated as a single independent locus with two alleles (present or absent). Unresolvable bands and missing data were scored as ambiguous for population genetic analyses. We removed individual isolates from further analyses if they produced AFLP profiles that were not consistent with standard profiles of *G. zeae* (<50% similarity) and if their morphological characteristics were not consistent with that of *G. zeae*. We cross-referenced a subset of the polymorphic AFLP bands segregating in the genetic linkage map of *G. zeae* (23), and have utilized the AFLP-locus naming terminology described in that study.

Genotype diversity assessments and population genetic analyses. AFLP haplotypes (putative clones) within and between heads and populations were identified by analyzing the binary data with the CLUSTER procedure of SAS (version 6.11 for Windows; SAS Institute, Cary, NC). The cluster analyses were used to identify single AFLP haplotypes both within and between heads in each population. Isolates with ≥98% (i.e., ≥92/94) AFLP band similarity were considered to belong to the same haplotype. We compared the frequency of recovery of *G. zeae* isolates and of individual AFLP haplotypes from heads in the two populations with the TTEST procedure of SAS with the null hypotheses that the frequencies of recovery were the same in both samples. A single isolate of each haplotype from each population was retained for genetic analyses of diversity and linkage disequilibrium.

We estimated allele frequencies of polymorphic loci and gene diversity within and between populations. We estimated the fixation index (G_{ST}) (29,37) as a measure of the extent of allelic differentiation between populations due to population subdivision relative to that of the total population; *Nm* (29) as an estimate of the relative amount of gene-flow among populations; and genetic identity among populations (38) with the shareware program Popgene version 1.21 (Molecular Biology and Biotechnology Centre, University of Alberta, Edmonton, Canada). The program was set to treat all AFLP data as haploid and dominant. G_{ST} and *Nm* were estimated with both the complete data set and with a subset of data that included only those loci for which both alleles were present at ≥5% frequency in at least one of the two populations. The results of these analyses were compared to determine whether including loci with rare alleles in these analyses altered the estimates of genetic differentiation of the populations.

Popgene also was used to estimate linkage disequilibrium for the 13/94 AFLP loci that were associated with a genetic locus on the linkage map (23), and for which both alleles were present in the population at a frequency of ≥5%. We calculated two-locus gametic disequilibria between all pairs of these 13 loci, and conducted χ^2 tests for significance as described by Weir (56).

RESULTS

Multilocus genotype distribution within populations. We isolated and examined 136 *Fusarium* isolates from the KS site and 140 *Fusarium* isolates from the ND site collected from 50 and from 54 heads, respectively. In both populations, the percentage of *G. zeae* isolates among the *Fusarium* isolates was high (>90%). A similar number of *G. zeae* isolates were recovered per head from the KS and ND sites (means of 2.26 and 2.22, respectively, *t* test, $P = 0.76$). No *Fusarium* isolates were recovered from three KS and four ND heads. Haplotype diversity was assessed from 123 KS and 130 ND *G. zeae* isolates utilizing a pool of 94 polymorphic AFLP loci (complete haplotype data for these strains available upon request).

We identified 79 and 90 multilocus haplotypes, respectively, from the KS and ND samples. In a subset of 26 isolates from 10

wheat heads, isolates in the same haplotype were always in the same VCG, although isolates in the same VCG could vary at one or two AFLP loci (≥98% unweighted pair group method with arithmetic means [UPGMA] similarity of their AFLP fingerprint profiles). The mean number of haplotypes recovered per head was 1.86 for the KS and 1.74 for the ND population, and did not differ statistically (t test, $P = 0.49$). These values probably underestimate the true means due to the small number of positions sampled on each head. Most haplotypes (74/79 from KS and 87/90 from ND) were recovered from only one head.

O'Donnell et al. (41) suggested that *G. zeae* populations causing Fusarium head blight of wheat and barley in the United States include only a single phylogenetic lineage: lineage 7. Our data indicate that all 253 tested *G. zeae* isolates have high AFLP haplotype similarity (>70%, UPGMA similarity) to one another and to reference strains of *G. zeae* lineage 7. We found no evidence to support the hypothesis that there is more than one phylogenetic lineage included in these Fusarium head blight populations.

Population divergence. Of the 94 scored AFLP loci, 85% were polymorphic in the KS population and 83% were polymorphic in the ND population (Table 1). Allele frequencies were generally very similar between these two populations (data not shown), as were the mean gene diversities (Table 1). Both alleles (presence and absence) were present at 68% (64/94) of the loci in both populations. There were 14 loci with private alleles (both allelic forms present in one population but not the other) in the KS population, and 16 such loci in the ND population (Table 1). For two of the loci with private alleles in the KS population the frequency of the private allele exceeded 5%. None of the frequencies of the private alleles in the ND population exceeded 5%. The mean frequency of the 30 private alleles across the two populations was <2.5%. When we removed all 43 loci with rare polymorphic alleles (frequency of the rarer allele <5% in both populations) from the analysis, the mean gene diversity estimates for both populations increased from ≈0.15 to ≈0.27 (Table 1).

For the full set of 94 loci, values of G_{ST}, for individual loci ranged from a minimum of 0 (i.e., no divergence or equal allele frequencies) to a maximum of 0.036. The mean G_{ST} (fixation index or differentiation among populations due to population subdivision) across all 94 loci was approximately 0.007, indicating that allele frequencies are very similar in the KS and ND populations (Table 2) with a correspondingly high *Nm* (the effective migration rate per generation) of ≈70. We obtained very similar results in the analysis of the subset of 51 loci for which the frequency of the rarer allele was >5% (mean $G_{ST} = 0.0071$ and $Nm = 70$) (Table 2). These results suggest that the KS and ND populations are both components of a larger, panmictic population.

TABLE 1. Summary of population statistical information comparing *Gibberella zeae* populations from Fusarium head blight epidemics at Kansas (KS) and North Dakota (ND) sites[a]

Population	KS	ND
Number of *G. zeae* isolates	123	130
Number of *G. zeae* haplotypes	79	90
Percent polymorphic loci	85	83
Number of private alleles	14	16
Mean frequency of private alleles[b]	0.025	0.018
Range	0.013–0.051	0.011–0.044
Mean gene diversity[b,c]		
94 loci	0.155	0.161
51 loci	0.270	0.277

[a] Up to three *G. zeae* isolates were recovered from each of upper, middle, and lower positions in 50, and 54 wheat heads in the KS (1993) and ND (1994) samples, respectively.
[b] Estimated for clone-censored populations. Clones were defined as isolates that shared ≥98% unweighted pair group method with arithmetic means similarity in amplified fragment length polymorphism banding pattern. Only one representative of each clone was retained in subsequent analyses.
[c] Calculated as in Nei (37).

Linkage disequilibrium. We used polymorphisms at 13 of the genetic loci that were mapped by Jurgenson et al. (23) to evaluate linkage disequilibrium in the KS and ND populations. These 13 loci are located on seven of the nine linkage groups of *G. zeae*: three loci were on each of linkage groups I and III, two loci were on linkage groups II and IX, and single loci were on each of linkage groups IV, VI, and VII (Table 3). The frequency of the rarer allele among these 13 loci ranged from 0.056 to 0.456. Allele frequencies at these loci were similar in both populations. Estimated G_{ST} values for these 13 loci ranged from 0 to 0.025 (Table 3), which was similar to the range observed for the full set of 94 loci (Table 2).

Of 78 possible pairwise comparisons between these 13 AFLP loci, only four locus pairs in the KS population and eight locus pairs in the ND population were in disequilibrium ($P < 0.05$) (Table 4). Three pairs of loci were estimated to be in disequilibrium in both the KS and ND populations (Table 4). Three of the four pairs of loci in linkage disequilibrium in the KS population involved a single AFLP locus (*ETGMTT0253K*) on linkage group IX. Two of the three loci on linkage group III (*EAAMAT0393K* and *ECCMCG0627K*) were not significantly out of equilibrium with each other, but were both in disequilibrium with *ETGMTT0253K*, $P = 0.039$ and 0.0002, respectively. In the ND population, three of the eight pairs of loci in disequilibrium also involved *ETGMTT0253K* (Table 4), and three more locus pairs included locus *EAAMAT0263K*, on linkage group I, which was in disequilibrium with single loci on linkage groups IV, VI, and IX ($P = 0.0188$, 0.0168, and 0.0265, respectively). Five of the eight pairs of loci in disequilibrium in the ND population involved loci on linkage group I. However, in only one instance did both members of a pair of loci that were in disequilibrium, *EAAMAT0765K* and *ETGMTT0328K* on linkage group I, map to the same linkage group ($P = 0.0026$). This pair of loci was the only pair located on the same linkage group that was in disequilibrium in either population.

DISCUSSION

Fusarium head blight epidemics in North America are sporadic and strongly correlated to local environmental conditions (17,44, 58). The populations analyzed in this study were distinct from one another in both space and time, and provide an opportunity to evaluate these local populations in a much broader context. Despite their separation in both time and distance, both populations were similar in their genetic diversity, in the mean number of *G. zeae* isolates per wheat head ($P > 0.75$), and in the mean number of AFLP haplotypes recovered per head ($P > 0.49$). The consistency between these two population samples suggests that similar populations could be found in other wheat fields in North America experiencing Fusarium head blight epidemics. Our findings generally are consistent with previous analyses of Fusarium

TABLE 2. Statistics on population genetic differentiation between Kansas and North Dakota clone-censored epidemic populations of *Gibberella zeae* calculated from all scored loci, and for the 51 loci for which the frequency of both alleles (presence and absence) was >5%

Statistic	94 Loci	51 Loci
Mean gene diversity[a]	0.159	0.276
Range	0.012–0.496	0.046–0.496
Fixation index (G_{ST})[a]	0.007	0.007
Range	0.0–0.036	0.0–0.036
Effective migration rate (Nm)[b]	70.5	69.9
Range	13.5–2000	13.5–2000
Genetic identity[c]	0.999	0.997

[a] Calculated as in Nei (37).
[b] Calculated as in McDermott and McDonald (29).
[c] Calculated as in Nei (38).

head blight populations that used VCGs (5,28,35) or random amplified polymorphic DNA markers (14,50,54).

Multilocus haplotypes. Using AFLP multilocus haplotypes, we found that individual wheat heads may be infected by multiple strains during an epidemic, but that adjacent heads usually are colonized by different *G. zeae* strains. This pattern suggests that most infections are initiated by single genotypes (presumably ascospores) during Fusarium head blight epidemics, but that some secondary infection also can occur. We cannot, however, rule out the possibility that genetically identical conidia or ascospores (produced by homothallic sexual reproduction) are responsible for the infections of adjacent heads, or for the multiple infections of a single head.

VCG data have been used previously to show that individual wheat or barley heads can be infected with multiple strains of *G. zeae* (6,28). Our data are consistent with the common hypothesis (25) that members of the same VCG in *G. zeae* are clones, because members of the same VCG have the same AFLP haplotype (i.e., are identical at ≥98% of the bands in the AFLP banding patterns). The number of vegetative incompatibility (*vic*) loci segregating in populations of *G. zeae* is not known, although in Kansas this number is at least five and likely to be much higher (5). In other ascomycete fungi (25), from 6 to 10 independent *vic* loci have been identified. Assuming only two *vic* alleles are found at each locus, six recombining loci can produce 64 (2^6) unique VCGs, and 10 such recombining loci produce >1,000 (2^{10}) unique VCGs. If there are more than two alleles, then the numbers are even larger (e.g., for three alleles at each of 10 loci there are >59,000 possibilities [3^{10}], and at four alleles at each of 10 loci >1,000,000 [4^{10}] possibilities). Thus, a large number of VCGs, each generally associated with a limited number of unique neutral marker genotypes, is expected in a population that undergoes relatively frequent sexual reproduction. The association between VCG and AFLP genotype observed in these *G. zeae* populations is consistent with the hypothesis that relatively frequent outcrossing is an important portion of the life cycle of this fungus. This hypothesis is not always true for *Fusarium* spp., however, because some researchers (12) have found that members of the same VCG in *F. verticillioides* have different AFLP haplotypes. Thus, AFLPs are preferred to VCGs for future studies of population diversity in *G. zeae* because they provide more detailed genotypes for analysis than do VCGs, are less laborious to generate, and do not require pairwise testing. If three pairs of AFLP primers are used to generate multilocus haplotypes, they have similar or greater power to identify genetic individuals (clones) than do VCG analyses.

Genetic diversity within populations. Two tools for managing Fusarium head blight are fungicide treatments or the deployment of resistant cultivars (3,30). In both cases, there is a risk that *G. zeae* populations could adapt to these control measures. Increased resistance to benzimidazole fungicides has been reported in China (10,19), and similar resistance could develop to triazole fungicides in the United States. Differences in isolate aggressiveness also have been reported in *G. zeae* (31,33,34), suggesting potential for further pathogenic adaptation and evolution. Although cultivar–isolate specificity has not been reported for the *G. zeae*–wheat interaction (31,32), Carter et al. (9) suggested that *G. zeae* from Nepal may be better adapted for either rice or maize as a host. However, the host specificity they describe also could be associated with phylogenetic lineage-specific differences (41) that had not been described at the time of the Carter et al. (9) study.

If sexual recombination is occurring between isolates of this homothallic fungus under field conditions, then new gene combinations for traits such as fungicide resistance or aggressiveness could be generated rapidly and dispersed in *G. zeae* populations. We found 79 haplotypes from 123 isolates in the KS population and 90 haplotypes from 130 isolates in the ND population. When Chen and McDonald (11) identified comparable genotypic diversity in populations of *Mycosphaerella graminicola*, they inferred that sexual reproduction played a significant role in those populations. Genotypic diversity in both epidemic populations in the present study was high, and our data are consistent with a hypothesis of relatively frequent outcrossing and sexual recombination in these field populations.

TABLE 4. Summary of the pairs of amplified fragment length polymorphism (AFLP) loci that show evidence of linkage disequilibrium ($P < 0.05$) in the Kansas and North Dakota populations

	Probability of equilibrium distribution[b]	
Locus 1/locus 2[a]	Kansas	North Dakota
EAAMAT0765K (I)/*ETGMTT0328K* (I)	NS	0.0026
ETGMTT0328K (I)/*EAAMAT0199K* (III)	NS	0.0453
EAAMAT0263K (I)/*ETGMTT0318K* (IV)	NS	0.0188
EAAMAT0263K (I)/*EAAMAT0500K* (VI)	NS	0.0168
EAAMAT0263K (I)/*ETGMTT0253K* (IX)	NS	0.0265
ECCMCG0627K (III)/*ETGMTT0514J* (VII)	0.0058	0.0066
EAAMAT0393K (III)/*ETGMTT0253K* (IX)	0.0393	NS
ECCMCG0627K (III)/*ETGMTT0253K* (IX)	0.0002	0.0059
ETGMTT0514J (VII)/*ETGMTT0253K* (IX)	0.0062	0.0308

[a] Roman numerals in parentheses = linkage group. AFLP locus nomenclature and linkage group assignments as described in Jurgenson et al. (23). All other pairwise estimates of linkage disequilibrium were nonsignificant at $P < 0.05$ in both populations.
[b] NS = not significant.

TABLE 3. Amplified fragment length polymorphism (AFLP) markers used in calculations of linkage disequilibrium and their assigned linkage groups, allele frequencies, and estimated G_{ST} statistics from the Kansas and North Dakota epidemic populations

		Frequency of AFLP presence allele[b]		
AFLP locus[a]	Linkage group	Kansas	North Dakota	G_{ST}[c]
EAAMAT0765K	I	0.620	0.708	0.0086
ETGMTT0328K	I	0.795	0.764	0.0014
EAAMAT0263K	I	0.644	0.578	0.0046
ETGMTT0395K	II	0.051	0.079	0.0031
ECCMCG0114K	II	0.766	0.621	0.0249
EAAMAT0393K	III	0.671	0.544	0.0168
ECCMCG0627K	III	0.895	0.944	0.0084
EAAMAT0199K	III	0.575	0.567	0.0001
ETGMTT0318K	IV	0.816	0.832	0.0004
EAAMAT0500K	VI	0.244	0.244	0.0000
ETGMTT0514J	VII	0.692	0.581	0.0133
ETGMTT0253K	IX	0.608	0.730	0.0170
ETGMTT0399K	IX	0.346	0.267	0.0069

[a] AFLP locus nomenclature follows, and linkage groups are designated as in Jurgenson et al. (23).
[b] Presence alleles are those alleles defined by an AFLP band presence character state.
[c] Calculated as described in Nei (37).

We observed little evidence for linkage disequilibrium in either population when we examined associations among the 13 mapped AFLP loci, with only 5% of the locus pairs examined in the KS population and 10% of the locus pairs examined in the ND population showing any evidence for disequilibrium at $P < 0.05$ (Table 4). Of the 12 locus pairs that are in disequilibrium in at least one of these two populations, 8 involve one, or both, of loci *ETGMTT0253K* or *EAAMAT0263K*. However, these data must be interpreted as preliminary in this respect, because Brown (8) notes that sample sizes of >100 individuals may be necessary to statistically and unambiguously detect disequilibria between some loci in natural populations. Thus, we cannot determine whether there is weak linkage disequilibrium within these populations, weak selection for a few specific allelic combinations, or whether the few observed disequilibria are statistical artifacts resulting from insufficient sample sizes. Our data indicate that North American populations are different from those described in China (19), in which approximately one-third of the locus pairs were in disequilibrium. Analysis of additional field populations from diverse geographic regions will be needed to provide an overall picture of the degree of genetic exchange within the global population of *G. zeae*, and to identify geographic regions or subpopulations that differ from the global population.

Perhaps the best evidence for regular recombination in these populations is that no loci on the same genetic linkage group are in disequilibrium in the KS population, and that only one pair of loci, on linkage group I, is in disequilibrium in the ND population (Table 4). These data, combined with the high levels of observed genotypic diversity, suggest that recombination has been occurring. These data are insufficient, however, to determine the temporal frequency with which outcrossing occurs, the percentage of the individuals that participate in the outcrossing events, or the fertility of such outcrosses relative to homothallic reproduction.

The pair of loci, *EAAMAT0765K* and *ETGMTT0328K*, on linkage group I that is in disequilibrium in the North Dakota population are closely linked (within 10 map units) to the *TRI5* locus, which is a part of a trichothecene biosynthetic gene cluster (23). Based on variation in DNA sequences, Ward et al. (55) hypothesized that recombination is reduced near the ends of this gene cluster. A reduction in recombination frequency also could lengthen the time that it takes for the alleles at adjacent loci to reach equilibrium, and could explain the disequilibrium observed for these loci in the North Dakota population. However, a more in-depth analysis of linkage disequilibrium on this portion of linkage group I is needed to test the Ward et al. (55) hypothesis at the level of local populations. The lack of disequilibrium at these loci in the Kansas population suggests that neither disruptive selection nor selection for a newly introduced allele in this portion of linkage group I has occurred in the Kansas population for quite some time.

Similarity between populations. Despite being collected in different years and from sites over 800 km apart, the low values for G_{ST} (<0.01) and an estimate of genetic identity very nearly equal to 1.0 (Table 2) both suggest that these two populations are part of a much larger, probably panmictic, pathogen population covering much of the central United States. Dusabenyagasani et al. (14) made similar estimates of both population diversity and interpopulation similarity for Canadian populations of *G. zeae* ($G_{ST} < 0.05$), suggesting that a similar conclusion can be reached for these populations, and perhaps for much of the wheat-growing region of North America. Low levels of G_{ST} also have been reported between populations of other plant-pathogenic fungi, including *Rhizoctonia* (47), *Cronartium* (15), and *Rhynchosporium* (48) spp. and, in these cases, the authors also concluded that these fungi exist as larger, well-mixed populations. The present study was limited to only two populations of *G. zeae* from the Great Plains. More intensive sampling of *G. zeae* populations from inside and outside the United States should allow us to more thoroughly test for barriers to gene flow in this fungus. Such barriers, if they exist, could be important for understanding Fusarium head blight epidemiology and for predicting the evolutionary potential of this pathogen.

Implications for Fusarium head blight epidemiology. *G. zeae* ascospores are thought to be the primary means of dispersal and infection in Fusarium head blight epidemics, (3,17,51). In this study, we found an average of ≈1.8 genotypes per head. This value almost certainly is an underestimate because we sampled only three positions within each head. The number of heads with no infections can be used to estimate the number of independent infections per head if infections on wheat heads occur randomly and independently, because the number of infections per head would then follow a Poisson distribution. The mean of such a distribution can be estimated from the class that has zero infections per head. For the Kansas population, 3/50 heads were not infected and, for the North Dakota population, 4/54 were not infected, which yields estimates of a mean of 2.8 and 2.6 infections per head, respectively. These numbers are approximately one order of magnitude lower than the daily colony counts of *G. zeae* per spike per day in an epidemic (17). Thus, the infection efficiency of *G. zeae* propagules on spikes may be low.

G. zeae populations that cause Fusarium head blight on wheat in the Great Plains region of the United States appear to be locally genetically diverse, participate in relatively frequent recombination, and are homogeneous across sampling years and large distances. The high interpopulation connectivity, illustrated by low G_{ST} values and high genetic identity between populations, and regular recombination both suggest that attempts to track local inoculum sources based on genetic signatures or allele frequency differences probably will fail. Also, the lack of differentiation between these populations for neutral genetic markers suggests that any differences in disease resistance ratings among locations within the region are unlikely to be due to genetic differences in the pathogen population. Frequent genetic recombination and gene flow in *G. zeae* populations means that novel or advantageous pathogenic traits that appear, whether they occur de novo by mutation or by migration from other geographic regions, could quickly be incorporated into diverse genetic backgrounds and may be dispersed rapidly throughout the region. Additional analysis of temporally and geographically separate populations is needed to evaluate the risks that immigrant strains and naturally occurring hybrids between phylogenetic lineages could pose to wheat in the United States.

Finally, the lack of population subdivision and the high genotypic diversity at small spatial scales may have implications for sampling field populations of *G. zeae*. We obtained very similar populations in samples from small 0.25-m^2 quadrats that were approximately 800 km apart. This result suggests that a variety of population sampling designs may be satisfactory for this fungus, at least in North America.

ACKNOWLEDGMENTS

Contribution no. 02-494-J from the Kansas Agricultural Experiment Station, Manhattan. Funding for this work came in part from the Kansas Agricultural Experiment Station and from the United States Wheat and Barley Scab Initiative grant 58-5430-2-327. We thank D. Wetzel, A. Beyer, and S. Hogarth for technical assistance; M. McMullen for collecting wheat samples from the North Dakota site; and K. O'Donnell for providing reference strains of the *G. zeae* lineages.

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